Hydroxylation products (compounds **3** and **4**) were major components in the liver extract. The metabolic pathway of famoxadone in chicken is presented in Fig 5.

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The sex-specific sulfation of the major metabolite of the novel fungicide cyprodinil in the rat

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Abstract: The metabolism of cyprodinil, a novel broad-spectrum fungicide, was investigated in rats. After single oral administration of 0.5 or 100 mg kg^{-1} body weight, [*phenyl-U-*^{14}C]cyprodinil was rapidly eliminated, principally in the urine. The metabolite pattern in urine exhibited a significant sex-related difference with respect to the major metabolite. Males and females both produced a dihydroxy metabolite, *N*-4-(hydroxyphenyl)-4-cyclopropyl-5-hydroxy-6-methylpyrimidin-2-ylamine. Female rats conjugated this metabolite with sulfate exclusively at the 5-hydroxypyrimidinyl moiety, while males formed equal amounts of the monosulfate and a disulfate conjugate. The sex dimorphism in the conjugation reaction indicates the involvement of a sex-specific sulfotransferase that catalyzed the transfer of the second sulfate group.

Keywords: cyprodinil; fungicide; metabolism; conjugation; sulfotransferase; sulfation; disulfate; sex dimorphism

1 INTRODUCTION

Cyprodinil, (4-cyclopropyl-6-methyl-*N*-phenylpyrimidin-2-ylamine), belongs to the novel class of pyrimidinamine fungicides. Cyprodinil exhibits a broad-spectrum activity against a variety of phytopathogenic fungi, making it suitable for protection of cereals, grapes, apples and vegetables.[1] The pyrimidinamines interfere with methionine biosynthesis of phytopathogenic fungi, a pathway specific to microorganisms and plants.[2] As part of the toxicological evaluation of cyprodinil, the urinary metabolites were identified after oral administration to rats.

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2 EXPERIMENTAL

[*Phenyl-U*-^{14}C] cyprodinil was administered by gastric intubation to two groups of young rats (Tif: RAI SPF, 195–215 g body weight), each consisting of five males and five females. One group of rats received a single low dose of 0.5 mg kg^{-1} body weight, while the rats of the second group received the high dose of 100 mg kg^{-1}. The test substance was dissolved in ethanol+polyethylene glycol 200+ water (1 + 2 + 1 by volume) and administered at *c*4 ml kg^{-1}. The rats were housed in metabolism cages throughout the study and permitted free access to food and water. The radioactivity in urine was determined by liquid scintillation counting. Faeces samples were homogenized manually with a pestle after addition of water (*c*1 ml), and aliquots were combusted in a Tri-Carb Sample Oxidizer (Packard). The 0–48 h urine was analysed by HPLC on a Zorbax ODS C18 column (4.6 mm ID × 250 mm) using a Beckman chromatography system connected to a Ramona A radioactivity flow monitor (Raytest). The solvent system was 10 mM ammonium formate + methanol (100 + 0 by volume) for 5 min then to 50 + 50 by volume over 40 min, to 10 + 90 by volume over 15 min, then held at this composition; the flow rate was 1 ml min^{-1}. The quantitative distribution of metabolites was determined by integration of the radioactivity detector signal using the Nelson Analytical Turbochrom® software. Metabolites present in urine were isolated by HPLC, applying successive chromatography on reverse-phase columns. Their structures were elucidated by [^{1}H]NMR and Fast Atom Bombardment mass spectroscopy.

3 RESULTS

The administered radioactivity was rapidly eliminated irrespective of the dose level and the sex of the animals. Within 48 h 92–97% of the dose had been excreted (Table 1). The principal route of elimination was the urine, since 53–60% of the dose was excreted in this over 168 h. Lower amounts were eliminated with the faeces (37–45%). Within seven days the administered radioactivity had been almost completely eliminated.

HPLC analysis of male rat urine revealed a metabolite pattern that was dominated by two metabolites, designated M1 and M2 (Fig 1). In contrast to that of males, the urine of female rats contained metabolite M1 but not M2. The distribution of both metabolites was independent of the dose level (Table 2). The combined metabolites M1 and M2 amounted to 30% of the dose in male rats, which was in the same range as the percentage of metabolite M1 alone in females (31–35%). The distribution of all other urinary metabolites (M3, M4, M5, M6 and M7) did not show a sex- or dose-related difference.

Cyprodinil is metabolized by sequential oxidation of the phenyl and pyrimidinyl rings (Fig 2). Hydroxylation of the phenyl or the pyrimidinyl ring yields the 4-hydroxyphenyl or 5-hydroxypyrimidinyl metabolites

Table 1. Excretion of radioactivity after single oral administration of [*phenyl-U-*[14]C]cyprodinil to rats

	Time after administration (h)	% of Dose (± SD)[a] 0.5 mg kg^{-1} Males	0.5 mg kg^{-1} Females	100 mg kg^{-1} Males	100 mg kg^{-1} Females
Urine	0–24	50.5 (±4.4)	53.9 (±5.4)	51.1 (±3.6)	56.3 (±1.9)
	24–48	1.6 (±0.4)	2.7 (±1.3)	1.9 (±0.6)	2.7 (±0.9)
	48–168	0.6 (±0.3)	1.4 (±0.9)	0.6 (±0.3)	0.7 (±0.3)
Subtotal		52.7 (±4.8)	58.0 (±6.1)	53.6 (±3.2)	59.7 (±1.7)
Faeces	0–24	40.2 (±4.8)	27.5 (±7.5)	37.2 (±1.6)	27.5 (±2.9)
	24–48	4.4 (±1.7)	8.0 (±3.0)	5.5 (±1.6)	8.3 (±3.1)
	48–168	0.7 (±0.5)	2.0 (±1.7)	0.8 (±0.3)	1.6 (±0.8)
Subtotal		45.3 (±4.4)	37.5 (±3.8)	43.5 (±2.7)	37.4 (±2.7)
Total excretion	0–168	98.0 (±1.2)	95.5 (±3.2)	97.1 (±1.1)	97.1 (±2.0)

[a] $n = 5$.

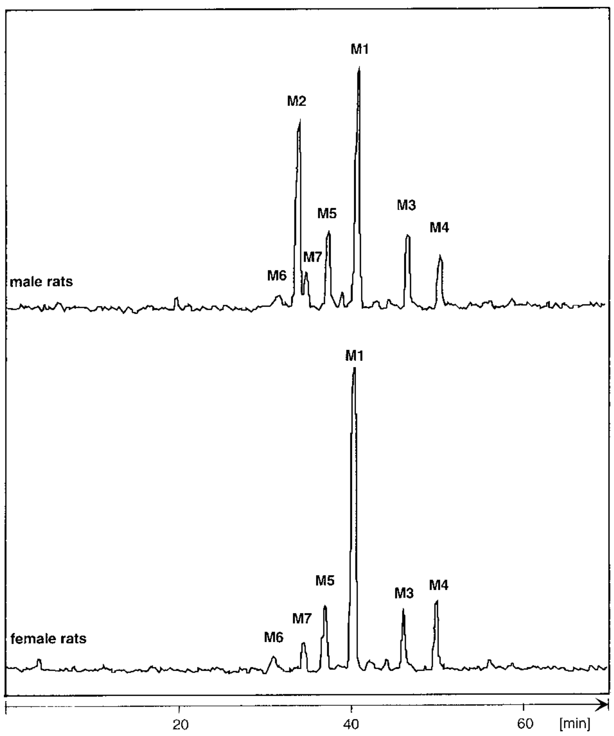

Figure 1. HPLC chromatogram of urinary metabolites of male and female rats administered a single oral dose of [*phenyl-U-*[14]C]cyprodinil at 100 mg kg^{-1}.

respectively, which are excreted as sulfate conjugates (M3, M4). A minor pathway is the further oxidation of the 4-hydroxyphenyl moiety to a 3,4-dihydroxyphenyl metabolite which is sulfated at the 3-hydroxy group (M5). The major pathway involves hydroxylation on both rings to form a 4-hydroxyphenyl-5-hydroxypyrimidinyl metabolite. Prior to excretion, the dihydroxy metabolite is conjugated with sulfate and to a lesser extent with glucuronic acid. Sulfation and glucuronidation of the 5-hydroxypyrimidinyl group, yielding metabolites M1 and M7 respectively, occurs to the same extent in male and female rats. However, in male rats the sulfated 5-hydroxypyrimidinyl metabolite undergoes further sulfation at the 4-hydroxyphenyl group, yielding the disulfate conjugate (M2) which is not found in females. Small amounts of a trihydroxy

Table 2. Quantitative distribution of metabolites in urine 0–48 h after single oral administration of [*phenyl-U-*[14]C]cyprodinil to rats

Metabolite	% of Dose 0.5 mg kg^{-1} Males	0.5 mg kg^{-1} Females	100 mg kg^{-1} Males	100 mg kg^{-1} Females
M1	17.2	34.7	17.0	31.0
M2	12.8	ND[a]	13.6	ND
M3	3.6	2.3	5.4	5.9
M4	5.1	5.9	7.6	8.2
M5	5.9	8.3	5.5	6.7
M6	3.3	2.3	1.9	2.5
M7	3.3	2.0	2.5	2.7

[a] ND: not detected.

metabolite (M6) are excreted unconjugated in males and females.

4 DISCUSSION

Disulfate conjugates are rarely observed in the metabolism of xenobiotics, but they are well known for endogenous dihydroxysteroids, eg. estra-3,17-diol, andro-5-ene-3,17-diol, 3,21-dihydroxy-5-pregnene-20-one.[3–5] A disulfate conjugate was found to be a major metabolite of diphenylamine in rat urine.[6] The sulfation of steroids is catalyzed by steroid sulfotransferases. In contrast, the dihydroxy-diarylamines, such as 4,4′-dihydroxydiphenylamine and the dihydroxy metabolite of cyprodinil, are likely to be substrates of phenol sulfotransferases.[7,8]

Two distinct sulfotransferases appear to be involved in the sulfation of the dihydroxy metabolite of cyprodinil in male rats. The first sulfotransferase mediates the transfer of sulfonate to the 5-hydroxypyrimidinyl site. The sulfation of the 4-hydroxyphenyl group is most likely catalyzed by a second sulfotransferase having a different specificity, because the polarity and hydrophilicity of this molecule are increased significantly with the insertion of the first sulfate group. The activity of the second sulfotransfer-

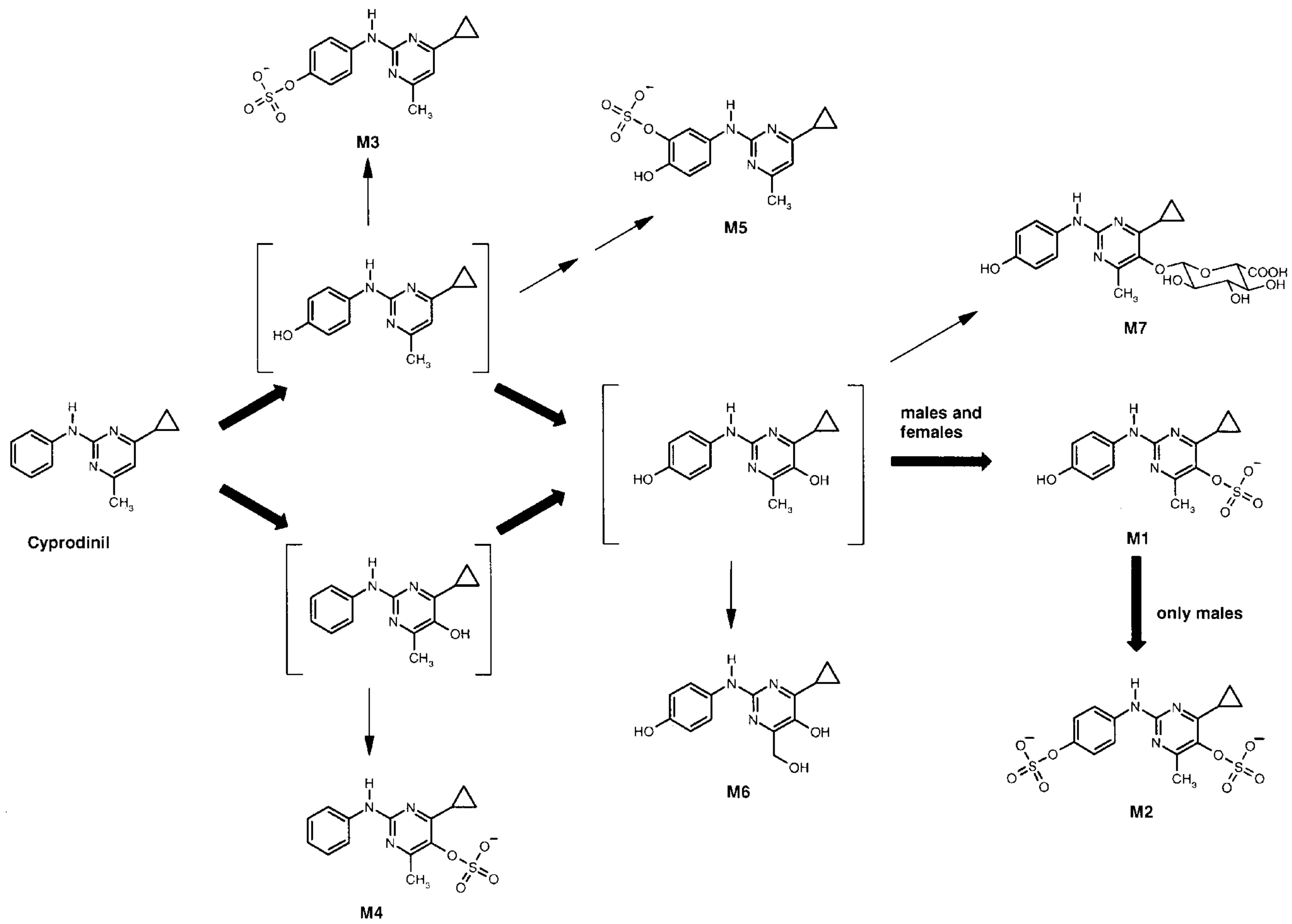

Figure 2. Major metabolic pathways of cyprodinil in the rat.

ase is sex-dependent, since only male rats formed the disulfate conjugate. Sex-related differences have been reported for the activity of some phenol sulfotransferases in rat liver.[9] For instance, large amounts of phenol sulfotransferase 1 were found for both sexes while large amounts of phenol sulfotransferase 2 were restricted to males.[10] However, it remains to be determined which sex-specific sulfotransferase is involved in the formation of the disulfate conjugate M2.

5 CONCLUSION

Cyprodinil is rapidly excreted, principally in the urine, after a single oral administration. Excretion of the administered dose is independent of the dose level and the sex. The major Phase I metabolites are conjugated with sulfate. Only male rats form a disulfate conjugate, suggesting a sex dimorphism in the activity of a specific sulfotransferase that catalyzes the transfer of the second sulfate group.

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The microbial biodegradation of paraquat in soil

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Abstract: The microbial degradation of [^{14}C]paraquat using cultures from two agricultural soils was investigated. The experiments were carried out in the absence of light, under aerobic conditions.

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